

Morphology of third instar *Boettcherisca highlandica* Kurahashi & Tan, 2009 (Diptera: Sarcophagidae)

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Abstract

Five species of *Boettcherisca* have been recorded hitherto in Malaysia. The present study demonstrates that *Boettcherisca highlandica* Kurahashi & Tan could be playing a role in forensic investigations as the larvae were recovered from a rabbit carcass placed at Cameron Highlands, peninsular Malaysia. The third instar morphology of *B. highlandica* is described for the first time. The larval length ranged from 17-18 mm, with the anterior spiracle composed of 28-30 papillae arranged in two irregular rows. The posterior spiracle is large, heavily pigmented with dilated tails at the upper and lower end of peritreme. Morphological differences of third instar and adults between *B. peregrina* and *B. highlandica* are highlighted, and keys to differentiate species of Sarcophagidae of forensic importance are provided herein.

Keywords: *Boettcherisca highlandica*, Sarcophagidae, third instar, forensic entomology, Cameron Highlands, Malaysia.

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Introduction

The literature on the species richness and biogeography of Sarcophagidae in Malaysia is relatively scarce compared to other dipteran families such as Calliphoridae. Sugiyama et al. (1990) described six new species of sarcophagine flies from Malaysia and Singapore. Twelve years later, a taxonomic key of the oriental Sarcophagidae was provided by Kurahashi (2002), where several new species were added. In 2007, Kurahashi & Leh listed the species of Diptera collected in Sarawak, East Malaysia and documented 21 species of Sarcophagidae, along with a new species described, *Parasarcophaga omari* Kurahashi & Leh, 2007. It was then followed by Kurahashi & Tan (2009) who published a checklist on the sarcophagid flies from peninsular Malaysia which comprised two subfamilies, 20 genera and 45 species. Soon after that, a sarcophagid,

Iranihindia martellata (Senior-White, 1924) was documented as a new locality record for peninsular Malaysia (Tan et al., 2010a).

The genus *Boettcherisca* was established by Rohdendorf in 1937 where he designated *Myophora peregrina* Robineau-Desvoidy, 1830 as type species. Lopes (1961) listed seven species in the checklist including *B. septentrionalis* Rohdendorf; *B. formosensis* Kiener & Lopes; *B. nathani* Lopes; *B. javanica* Lopes, *B. peregrina* (Robineau-Desvoidy); *B. karnyi* (Hardy) and *B. atypica* (Baranov). The phylogeny of *Boettcherisca* can be divided into two monophyletic and one paraphyletic group, namely *peregrina*-group, *septentrionalis*-group and *karnyi*-group, respectively. Based on the cladogram analysis and geographic distribution, the genus *Boettcherisca* is postulated to have a Sundaland origin (Kurahashi & Kano, 1984).

Recently, a total of 17 species of *Boettcherisca* were recorded by Xue et al. (2011), where most of the species are distributed in Oriental region. The oriental species include *B. bengalensis* Nandi; *B. cabrerai* Kano & Sugiyama; *B. dumoga* Sugiyama & Kurahashi; *B. highlandica*; *B. javanica*; *B. koimani* Kano & Shinonaga; *B. krathonmai* (Pape & Bänzinger); *B. nepalensis* Kano & Sugiyama; *B. talomensis* Magpayo & Kano; *B. timorensis* Kano & Shinonaga; and *B. yuwanensis* (Sugiyama). The remaining six species of *Boettcherisca* have a wider geographical distribution viz., *B. formosensis* (Palearctic, Oriental); *B. invaria* (Walker)(= *atypica* Baranov) (Oriental, Australasian/Oceanian); *B. karnyi* (Oriental, Australasian/Oceanian); *B. nathani* (Palearctic, Oriental); *B. peregrina* (Palearctic, Oriental, Australasian/Oceanian, Madagascar); and *B. septentrionalis* (Palearctic, Oriental).

In peninsular Malaysia, five species of *Boettcherisca* have been previously recorded. These included *B. peregrina*, *B. karnyi*, *B. javanica*, *B. krathonmai* and *B. highlandica* (Kurahashi & Tan, 2009). However, only four species were documented in Sarawak (Malaysian Borneo), with no record of *B. highlandica* (Kurahashi & Leh, 2007).

The biology of *B. peregrina* is relatively well documented. The adult flies of *B. peregrina* are extensively distributed in eusynanthropic as well as the semisynanthropic and asynanthropic zones on human feces especially in latrine areas (Feng et al., 1990). The adult flies feed on garbage, corpses, feces, flowers and fallen fruits (Xue et al., 2011). The larvae of *B. peregrina* can be found in a wide variety of decomposing organic matter such as carrion, dung, garbage and human feces (Hall & Bohart, 1948; Lopes, 1961), including dead giant African snail, *Achatina fulica* Bowdich (Senior-White et al., 1940, as *Sarcophaga fuscicauda* Boettcher). However, dead snails are probably not the primary breeding site for *B. peregrina*. According to Beaver (1986), *B. peregrina* rarely breeds on *A. fulica* in Thailand, and in three out of five experiments in Malaysia, but only in a single snail in each experiment. The numbers of individuals bred per snail varied from 1-6, and adults emerged from 15-19 days after exposure of the snails. Other than snails, the larvae have

been recovered from dead common Indian toad (*Bufo melanostictus*) (Das & Dasgupta, 1986). Furthermore, adults have been reared from candle bush (*Cassia alata* L.)(= *Senna alata* (L.) Roxburgh) and on "rotting shells" (Lopes, 1961). The larvae can be parasites of sugarcane looper, *Mocis frugalis* (Fabricius) (Mungomery, 1947), earthworms, and locust, *Chortoicetes terminifera* (Walker) and act as facultative predators of lepidopteran pupae of nymphalid and pyralid (Xue et al., 2011).

Previous studies demonstrated that *B. peregrina* breeds on human and animal remains (Sukontason et al., 2010) and served as an causative agent in various kind of myiasis such as ophthalmomyiasis (Miura et al., 2005), otomyiasis (Chigusa, 1994), cutaneous myiasis (Kani et al., 1981), nasal myiasis (Kamimura & Arakawa, 1986), intestinal myiasis (Hasegawa et al., 1992), urogenital myiasis (Jiang, 2002), vaginal myiasis (Chigusa et al., 2005) and urethral myiasis (Sun & Ren, 1995). Furthermore, this species was reported in hospital-acquired myiasis (Uni et al., 2006). In forensic entomological studies, Early & Goff (1986) recovered the larvae of *B. peregrina* from the exposed carrion on the island of Oahu, Hawaiian islands, USA, as well as on human remains found indoor in Hawaii (Frost et al., 2010). Besides, the larvae of *B. peregrina* have been used in entomotoxicology studies to determine the effect of drug substances on its developmental rates (Goff et al., 1989; Goff et al., 1991). From the medical perspectives, the adults have been incriminated as mechanical vectors of etiological pathogens of diseases acquired from decaying animal matter and human feces (Moribayashi et al., 2001). In molecular taxonomy, molecular profiles of forensically important flesh flies (which include *Boettcherisca* spp.) in Malaysia have been studied thoroughly by Tan et al. (2010b). Besides, the basic life cycle and life history of *B. peregrina* have been investigated by several researchers (Nishida et al., 1986; Majumder et al., 2012), including a study on its developmental rate under different temperature conditions (Wang et al., 2010).

However, there is little information on the biology and ecology of *B. highlandica*. The type localities of *B. highlandica* were Genting

Highlands and Cameron Highlands (both located in peninsular Malaysia) and this species appeared to be only present at high elevations (i.e., above 1,200 m a.s.l.) (Kurahashi & Tan, 2009). In this study, we had a chance to collect and examine several *B. highlandica* larvae obtained from a rabbit carcass placed in Cameron Highlands. The third instar morphology of *B. highlandica* is herein described for the first time.

Materials and Methods

A field experiment was conducted at Cameron Highlands (4.49N, 101.39E, 1,517.3 m a.s.l.). Observations and insect collections were carried out for 22 days continuously. A total of five rains were recorded during the study period, with mean temperature and relative humidity $23.56 \pm 4.04^{\circ}\text{C}$ and $81.73 \pm 21.56\%$, respectively. A rabbit (*Oryctolagus cuniculus* (L.)), weighted approximately 2.0 kg, was euthanized by phenobarbital overdose and then placed on the ground at the study site on Day 1 (initial day of study). The carcass was then surrounded by protective fence to prevent scavenging activities by vertebrates.

Three third instar larvae from a same maggot mass were collected beneath the carcass on the last day of observation (on day-22 postmortem). Two of them were preserved in 70% ethanol immediately on-site and the other one was allowed to grow by feeding it with beef liver and brought back to the laboratory for rearing purposes. The food source (i.e., beef liver) was added ad-libitum until the post-feeding stage, and then the larva was transferred to a dry container for pupation. The resulted adult fly was then killed by using Ether, pinned, and kept in the oven for desiccation over four days. The dried specimen was subsequently labeled and sent to the last author for species confirmation.

The preserved larvae were observed microscopically and the body length was measured by an ordinary ruler under a dissecting microscope (Olympus SZX10, Japan). Larval mouthparts, anterior spiracles, posterior spiracles and other morphological features were then prepared by using slide mounting methods modified from Lee et al. (2004) as follows: a transverse excision was made at eleventh body

segment without separating it into two parts. The excised larvae were placed in 10% potassium hydroxide (KOH) for clearing purposes. Immersion in KOH was continued overnight. The next day, they were placed in 10% acetic acid for five minutes to neutralize the KOH solution. Forceps, pins and a half-shaved wooden stick were used to aid in removing the gut contents of the larvae. The larvae were placed through a series of ascending concentrations of alcohol solutions from 70%, 90%, 95% and absolute alcohol for 30 minutes each. They were then placed in xylene for 7 minutes and in clove oil for 30 minutes for colouring purpose. Lastly, the larvae were placed on glass slides and mounted with Canada Balsam and kept in an oven for drying process. The slides were labeled and observed under a compound stereoscope (Olympus BX53, Japan) with a standardized measurement bar according to the respective magnifications. The pinned adult specimen and larval slides were vouchered in the parasitology laboratory, Faculty of Medicine, Universiti Teknologi MARA.

Results and Discussions

The first sarcophagid that arrived on the rabbit carcass was *Parasarcophaga albiceps* (Meigen) and it was observed on the third day of decomposition. Two days later, a male and a female *B. highlandica* were noted on the carcass, indicating a temporal delay in this species to locate ephemeral resources (Table 1). Adults of *B. highlandica* were sighted continuously until day-13 of decomposition. On the other hand, *Parasarcophaga taenionota* (Wiedemann) was also recovered at the later stage of decomposition. However, neither *P. albiceps* nor *P. taenionota* larviposited on rabbit carcass, although the adults were seen frequently on the carrion. The complete faunal successional data on rabbit carcasses at Cameron Highlands can be retrieved from Silahuddin et al. (2015).

We did not record the date and time of the initial larviposition by *B. highlandica* on the rabbit carcass, the developmental time from egg to larva stage could not be precisely determined. However, the duration needed from initial pupation stage to emergence of adult was noted under a well-controlled laboratory condition with a recorded pupariation time. The pupa was

formed on 3.II.2012 and the adult emerged on 20.II.2012. Hence the pupal duration was 18 days (~432 hours) under the laboratory condition ($23.7 \pm 0.17^{\circ}\text{C}$, $83.6 \pm 1.82\%$ RH, 10 h light: 14 h dark cycle). This preliminary data was based on a single emerged adult, therefore the duration of pupal stage might vary as there was no replication. It is necessary to study the complete development duration of *B. highlandica* as this species could have ecological implications and forensic value especially in the ecoregion of montane forest.

Description: third instar *B. highlandica* (n=2). **General:** length from 17-18 mm, body slender-shaped, creamy white in color. **Cephalopharyngeal skeleton:** medium sized and heavily pigmented; basal piece of mouth hook rectangular, hook part short, stout and pointed downward slightly; ventral cornua approximately half length of dorsal cornua; anterodorsal processes inapparent. **Anterior papillae:** arranged in two rows with 28-30 papillae. **Body spination:** each segment covered by spinations; the spine is of single spike (unicuspid), long, slender, sharp pointed at tip

and wholly pigmented. **Posterior spiracle:** large and heavily pigmented; peritreme incomplete with well developed inner slit projections; button absent; spiracular slits slender and long; distance between both posterior spiracles is approximately $\frac{3}{4}$ width of spiracle; tails of the upper and lower end of peritreme dilated (Figs. 1 and 2).

Comparative remarks. Since both *B. peregrina* and *B. highlandica* are carrion breeders, we provide taxonomic keys adapted from Sukontason et al. (2010) to distinguish these species and prevent misidentification in the future. We hereby highlighted some morphological differences between *B. peregrina* and *B. highlandica* larvae (Table 2) and adult stage (Table 3). The differences in their bionomics are also compared in Table 4.

We hope the information provided in this paper could be useful to forensic communities where *B. highlandica* could potentially serve as a forensic indicator in the determination of minimum postmortem interval and the location of body recovery.

Table 1. Succession of Sarcophagidae on a rabbit carcass placed at Cameron Highlands, Malaysia

Species recovered	Adult sighted on	Larval recovered from rabbit carcass
<i>Parasarcophaga albiceps</i> (Meigen)	Day 3	No
<i>Boettcherisca highlandica</i> Kurahashi & Tan	Day 5-13	Yes (larvae collected on Day-22 postmortem)
<i>Parasarcophaga taenionota</i> (Wiedemann)	Day 12-18	No

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Table 2. Larval morphology between *B. peregrina* and *B. highlandica*

Key features of third instar larva	<i>Boettcherisca peregrina</i> (adapted from Sukontason et al., 2010)	<i>Boettcherisca highlandica</i>
Cephalopharyngeal skeleton	Apparent anterodorsal processes	Inapparent anterodorsal processes
Anterior spiracle	Arranged in two rows with 24-26 papillae	Arranged in two rows with 28-30 papillae
Body spination	The spine is of single spike, long, slender, sharp pointed at tip and wholly pigmented	The spine is of single spike, long, slender, sharp pointed at tip and wholly pigmented
Posterior spiracle	Tail of the upper end of peritreme is dilated	Tails of the upper and lower ends of peritreme are dilated
Length of third instar larva	17 mm	17-18 mm

Table 3. Adult morphology between *B. peregrina* and *B. highlandica*

Key features of the adult	<i>Boettcherisca peregrina</i> (adapted from Sukontason et al., 2010)	<i>Boettcherisca highlandica</i> (adapted from Kurahashi & Tan, 2009)
Body length	10-12 mm	10-12 mm
Gena	Black	Black
Third antenna segment	Black	Black
Palpus	Black or dark brown	Black
Postsutural ac	Present	Present
Abdomen	Grey pollinosity at least on tergite 4-5 in male	Golden yellow pollinosity at least on tergite 4-5
Epandrium	Brown, occasionally blackish or reddish	Reddish brown

Table 4. Natural history between *B. peregrina* and *B. highlandica*

Significant natural history	<i>Boettcherisca peregrina</i>	<i>Boettcherisca highlandica</i>
Medical and forensic importance	Both myiasis and forensic cases were reported from human and animal carcasses	No myiasis or forensic case yet to be reported from human corpses. The larvae were recovered from animal carcass at high elevation, indicating its potential role in decomposition at the highland
Habitat	Low land (highest altitude recorded was 1,300 m a.s.l.) (Xue et al., 2011) secondary forest, rural and urban settling, plantations	Highlands with montane forest more than 1,200 m above sea level
Geographical distribution	Peninsular Malaysia and Malaysian Borneo, Singapore, Bangladesh, Bhutan, Laos, Myanmar, Nepal, Vietnam, Cambodia, Thailand, Indonesia, Japan, China, Taiwan, North Korea, South Korea, India, Sri Lanka, Seychelles, New Guinea, New Britain, French Polynesia, Fiji, Samoa, Volcano Island, Bonin Island, Gilbert Island, Guam, Kiribati, Hawaii and Mariana Island, Papua New Guinea, Australia, New Zealand (Kurahashi & Kano, 1984; Xue et al., 2011)	Peninsular Malaysia (Genting Highlands and Cameron Highlands)
Ecology	Synantrophic and widely distributed. Larvae are necrophagous and adults are coprophilous (Heo et al., 2010)	Asynantrophic. Larvae breed on carrion at high elevation

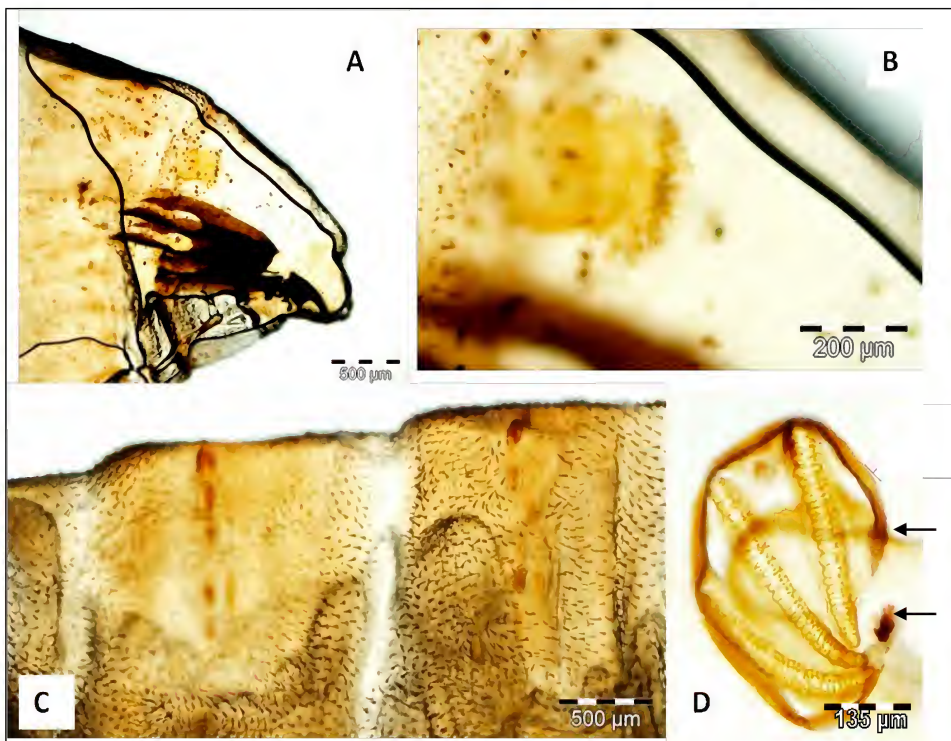


Fig. 1. Microscopic morphology of third instar *B. highlandica*. A, Cephalopharyngeal skeleton. B, Anterior spiracle with 28-30 papillae arranged in two irregular rows. C, Body spines with single spike (unicuspid). D, Left posterior spiracle. Peritreme with dilated upper and lower ends (arrows)

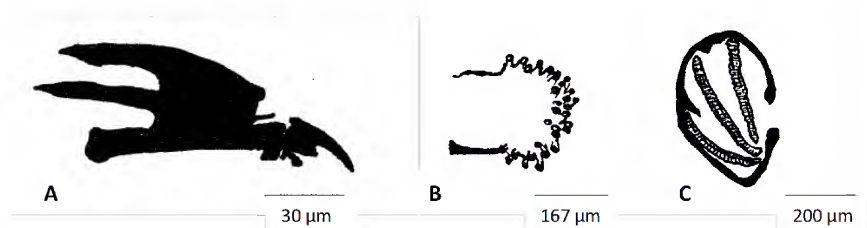


Fig. 2. Internal structures of third instar *B. highlandica*. A, Cephalopharyngeal skeleton. B, Anterior spiracle. C, Left posterior spiracle

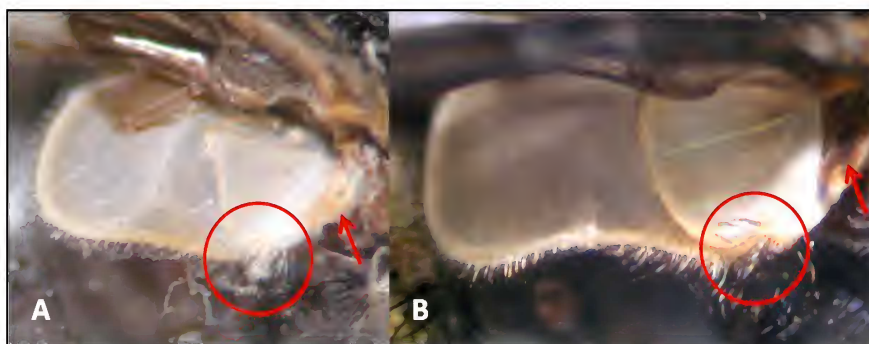


Fig. 3. Alar and thoracic squamae. A, *Boettcherisca peregrina*. B, *Boettcherisca highlandica*. Circle showed lower margin of alar squama. Arrow showed base of alar squama

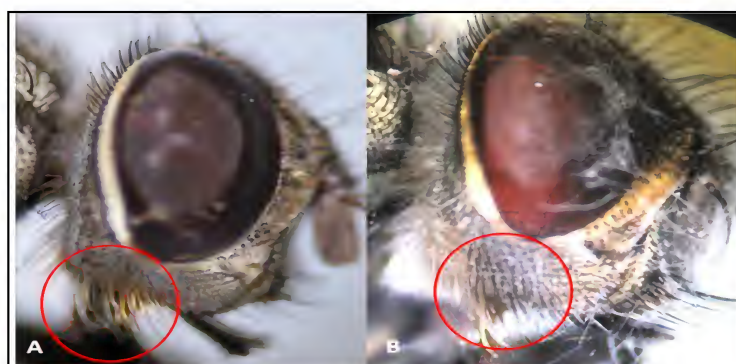


Fig. 4. Lateral view of head. A, *Boettcherisca peregrina*, gena clothed with yellowish hairs posteriorly. B, *Boettcherisca highlandica*, gena clothed with black hairs. Circle showed the gena region

Key to adult sarcophagids of forensic importance in Malaysia

1. Propleuron bare.....2
- Propleuron hairy.....3
2. Third antennal segment largely orange; palpus wholly orange; epandrium orange; postsutural ac absent.....***Liopygia ruficornis* Fabricius**
- Third antennal segment largely fuscous black; palpus wholly dark brown; epandrium brown; postsutural ac present.....***Parasarcophaga (Liosarcophaga) dux* Thomson**

3. Inner lower margin of alar squama and outer lower margin of thoracic squama with tuft of pale white hairs (Fig. 3A); gena clothed with yellowish hairs posteriorly (Fig. 4A).....***Boettcherisca peregrina* (Robineau-Desvoidy)**
- Inner lower margin of alar squama and outer lower margin of thoracic squama with tuft of fuscous black hairs (Fig. 3B); gena clothed with black hairs (Fig.4B).....***Boettcherisca highlandica* Kurahashi & Tan**

Key to third instar of sarcophagids of forensic importance in Malaysia (parts of the key were adapted from Sukontason et al., 2010)

1. Marginal papillae of anterior spiracle arranged in one regular row, composed of 11-17 papillae.....2
- Marginal papillae of anterior spiracle arranged in two or more irregular rows; composed of 24-30 papillae.....3
2. Anterior spiracle with 11-15 papillae; inter-slit projection distinct; lower end of peritreme located near base of middle or upper slit.....***Liopygia ruficornis* Fabricius**
- Anterior spiracle with 14-17 papillae; inter-slit projection indistinct; lower end of peritreme located near base of lowest slit...***Parasarcophaga (Liosarcophaga) dux* Thomson**
3. Anterior spiracle with 24-26 papillae; only tail of the upper end of peritreme dilated.....***Boettcherisca peregrina* (Robineau-Desvoidy)**
- Anterior spiracle with 28-30 papillae; tails of the upper and lower ends of peritreme dilated.....***Boettcherisca highlandica* Kurahashi & Tan**

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